

CLAIM AMENDMENTS

1. (Original) A method for inhibiting proliferation of a tumor in a mammal, the method comprising: administering to the mammal harboring the tumor a composition comprising,

- (a) an immunogenic stress protein-peptide complex isolated from a cell derived from the tumor, said complex being operative to initiate in the mammal an immune response against said tumor, and
- (b) a pharmaceutically acceptable carrier,

in an amount sufficient to elicit in the mammal an immune response against the tumor thereby inhibiting proliferation of the tumor.

19. (Currently amended) An isolated population of immunogenic human stress protein-peptide complexes isolated from human tumor tissue excised from a human, wherein ~~the peptides are noncovalently associated with the stress protein, and wherein the stress protein is human gp96~~ said complexes each comprise human gp96 noncovalently associated with a peptide.

20. (Currently amended) An isolated population of immunogenic human stress protein-peptide complexes isolated from human tumor tissue excised from a human, wherein said population of complexes is a combination of Hsp70-peptide complexes, Hsp90-peptide complexes, and gp96-peptide complexes; and wherein ~~the peptides are noncovalently associated with the stress protein~~ said complexes each comprise a stress protein noncovalently associated with a peptide.

21. (Currently amended) A composition comprising:

- (a) a therapeutically effective amount of purified immunogenic human stress protein-peptide complexes isolated from human tumor tissue excised from a human, wherein ~~the peptide is noncovalently associated with the stress protein, and wherein the stress protein is human gp96~~ said complexes each comprise gp96 noncovalently associated with a peptide; and
- (b) a pharmaceutically acceptable carrier.

22. (Currently amended) A method for treating a mammal having a tumor sensitive to treatment with a human gp96-peptide complex comprising administering to the mammal a composition comprising:

- (a) an amount of purified immunogenic human gp96-peptide complexes isolated from human tumor tissue excised from a human, wherein the

amount is sufficient to elicit an immune response against the tumor, wherein ~~the peptides are noncovalently associated with the gp96~~ said complexes each comprise gp96 noncovalently associated with a peptide; and

- (b) a pharmaceutically acceptable carrier.

23. (Added by first preliminary amendment) The method of claim 22 wherein the mammal is a human.

24. (Added by first preliminary amendment) The method of claim 23 wherein the mammal is the human from which the complexes are isolated.

25. (Currently amended) A method for treating a mammal having a tumor scnsitive to treatment with a human gp96 peptide complex comprising:

- (a) isolating immunogenic human gp96-peptide complexes from human tumor tissue excised from a human, wherein ~~the peptides are noncovalently associated with the gp96~~ said complexes each comprise gp96 noncovalently associated with a peptide; and
- (b) administering a composition comprising an amount of the isolated complexes sufficient to elicit an immune response against the tumor, and a pharmaceutically acceptable carrier.

26. (Added by first preliminary amendment) The method of claim 25 wherein the mammal is a human.

27. (Added by first preliminary amendment) The method of claim 26 wherein the mammal is the human from which the complexes are isolated.

28. (Currently amended) A method for eliciting in a mammal an immune response against a tumor comprising administering to the mammal a composition comprising:

- (a) an amount of purified immunogenic human gp96-peptide complexes isolated from human tumor tissue excised from a human, wherein the amount is sufficient to elicit an immune response against the tumor, wherein ~~the peptides are noncovalently associated with the gp96~~ said complexes each comprise gp96 noncovalently associated with a peptide; and
- (b) a pharmaceutically acceptable carrier.

29. (Added by first preliminary amendment) The method of claim 28 wherein the mammal is a human.

30. (Added by first preliminary amendment) The method of claim 29 wherein the mammal is the human from which the complexes are isolated.

31. (Added by first preliminary amendment) The method of claim 23, 24, 26, 27, 29 or 30 wherein the complexes are administered to the human in an amount in the range of 1 to 1000 micrograms of complex per kg body weight of the human per administration.

32. (Added by first preliminary amendment) The method of claim 23, 24, 26, 27, 29 or 30 wherein the complexes are administered to the human in an amount in the range of 100 to 250 micrograms of complex per kg body weight of the human per administration.

33. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 19, wherein said complexes are isolated using Concanavalin A affinity chromatography.

34. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 19, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

35. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 34, wherein the Concanavalin A is affixed to agarose beads.

36. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 34, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

37. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 34, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

38. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 19, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and
- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

39. (New) The composition of claim 21, wherein said complexes are isolated using Concanavalin A affinity chromatography.

40. (New) The composition of claim 21, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

41. (New) The composition of claim 40, wherein the Concanavalin A is affixed to agarose beads.

42. (New) The composition of claim 40, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

43. (New) The composition of claim 40, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

44. (New) The composition of claim 21, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and
- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

45. The method of claim 22 or 28, wherein said complexes are isolated using Concanavalin A affinity chromatography.

46. (New) The method of claim 22 or 28, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

47. (New) The method of claim 46, wherein the Concanavalin A is affixed to agarose beads.

48. (New) The method of claim 46, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

49. (New) The method of claim 46, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

50. (New) The method of claim 22 or 28, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and
- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

51. The method of claim 25, wherein said isolating step comprises Concanavalin A affinity chromatography.

52. (New) The method of claim 25, wherein said isolating step comprises:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

53. (New) The method of claim 52, wherein the Concanavalin A is affixed to agarose beads.

54. (New) The method of claim 52, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

55. (New) The method of claim 52, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

56. (New) The method of claim 25, wherein said isolating step comprises:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and
- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

57. (New) The composition of claim 21, further comprising an adjuvant.

58. (New) The composition of claim 57, wherein the adjuvant is selected from the group consisting of pluronic tri-block copolymer, muramyl dipeptide, detoxified endotoxin, saponin, and liposome.

59. (New) The composition of claim 57, wherein the adjuvant is QS-21.

60. (New) The method of claim 22, 25, or 28, wherein the composition further comprises an adjuvant.

61. (New) The method of claim 60, wherein the adjuvant is selected from the group consisting of pluronic tri-block copolymer, muramyl dipeptide, detoxified endotoxin, saponin and liposome.

62. (New) The method of claim 60, wherein the adjuvant is QS-21.
63. (New) An isolated population of immunogenic human stress protein-peptide complexes isolated from human tumor cells isolated from a human, wherein said complexes each comprise human gp96 noncovalently associated with a peptide.
64. (New) An isolated population of immunogenic human stress protein-peptide complexes isolated from human tumor cells isolated from a human, wherein said population of complexes is a combination of Hsp70-peptide complexes, Hsp90-peptide complexes, and gp96-peptide complexes; and wherein said complexes each comprise a stress protein noncovalently associated with a peptide.
65. (New) A composition comprising:
- (a) a therapeutically effective amount of purified immunogenic human stress protein-peptide complexes isolated from human tumor cells isolated from a human, wherein said complexes each comprise gp96 noncovalently associated with a peptide; and
 - (b) a pharmaceutically acceptable carrier.
66. (New) A method for treating a mammal having a tumor sensitive to treatment with a human gp96-peptide complex comprising administering to the mammal a composition comprising:
- (a) an amount of purified immunogenic human gp96-peptide complexes isolated from human tumor cells isolated from a human, wherein the amount is sufficient to elicit an immune response against the tumor, wherein said complexes each comprise gp96 noncovalently associated with a peptide; and
 - (b) a pharmaceutically acceptable carrier.
67. (New) The method of claim 66 wherein the mammal is a human.
68. (New) The method of claim 67, wherein the mammal is the human from which the complexes are isolated.
69. (New) A method for treating a mammal having a tumor sensitive to treatment with a human gp96 peptide complex comprising:
- (a) isolating immunogenic human gp96-peptide complexes from human tumor cells isolated from a human, wherein said complexes each comprise gp96 noncovalently associated with a peptide; and

- (b) administering a composition comprising an amount of the isolated complexes sufficient to elicit an immune response against the tumor, and a pharmaceutically acceptable carrier.
- 70. (New) The method of claim 69 wherein the mammal is a human.
- 71. (New) The method of claim 70 wherein the mammal is the human from which the complexes are isolated.
- 72. (New) A method for eliciting in a mammal an immune response against a tumor comprising administering to the mammal a composition comprising:
 - (a) an amount of purified immunogenic human gp96-peptide complexes isolated from human tumor cells isolated from a human, wherein the amount is sufficient to elicit an immune response against the tumor, wherein said complexes each comprise gp96 noncovalently associated with a peptide; and
 - (b) a pharmaceutically acceptable carrier.
- 73. (New) The method of claim 72 wherein the mammal is a human.
- 74. (New) The method of claim 73 wherein the mammal is the human from which the complexes are isolated.
- 75. (New) The method of claim 67, 68, 70, 71, 73 or 74 wherein the complexes are administered to the human in an amount in the range of 1 to 1000 micrograms of complex per kg body weight of the human per administration.
- 76. (New) The method of claim 67, 68, 70, 71, 73, or 74, wherein the complexes are administered to the human in an amount in the range of 100 to 250 micrograms of complex per kg body weight of the human per administration.
- 77. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 63, wherein said complexes are isolated using Concanavalin A affinity chromatography.
- 78. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 63, wherein said complexes are isolated by a process comprising:
 - (a) lysing cells of the tumor tissue to provide a lysate;

- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

79. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 68, wherein the Concanavalin A is affixed to agarose beads.

80. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 78, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

81. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 34, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

82. (New) The isolated population of immunogenic human stress protein-peptide complexes of claim 63, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and
- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

83. (New) The composition of claim 65, wherein said complexes are isolated using Concanavalin A affinity chromatography.

84. (New) The composition of claim 65, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

85. (New) The composition of claim 84, wherein the Concanavalin A is affixed to agarose beads.

86. (New) The composition of claim 84, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

87. (New) The composition of claim 84, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

88. (New) The composition of claim 65, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and

- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

89. The method of claim 66 or 72, wherein said complexes are isolated using Concanavalin A affinity chromatography.

90. (New) The method of claim 66 or 72, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

91. (New) The method of claim 90, wherein the Concanavalin A is affixed to agarose beads.

92. (New) The method of claim 90, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

93. (New) The method of claim 90, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

94. (New) The method of claim 66 or 72, wherein said complexes are isolated by a process comprising:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;

- (e) washing the agarose beads with a buffer; and
- (f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

95. The method of claim 69, wherein said isolating step comprises Concanavalin A affinity chromatography.

96. (New) The method of claim 69, wherein said isolating step comprises:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate to provide a clarified supernatant;
- (c) contacting the supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes in the supernatant are bound to Concanavalin A; and
- (d) eluting said complexes with a buffer comprising α -methyl mannoside.

97. (New) The method of claim 96, wherein the Concanavalin A is affixed to agarose beads.

98. (New) The method of claim 96, wherein said eluting step comprises washing with a buffer comprising 10% α -methyl mannoside.

99. (New) The method of claim 96, wherein said centrifuging comprises centrifuging the lysate at 1000 x g to provide a first supernatant and centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant.

100. (New) The method of claim 69, wherein said isolating step comprises:

- (a) lysing cells of the tumor tissue to provide a lysate;
- (b) centrifuging the lysate at 1000 x g to provide a first supernatant;
- (c) centrifuging the first supernatant at 100,000 x g to provide a clarified supernatant;
- (d) contacting the clarified supernatant with Concanavalin A under conditions such that the human stress protein-peptide complexes are bound to Concanavalin A, wherein said Concanavalin A is affixed to agarose beads;
- (e) washing the agarose beads with a buffer; and

(f) eluting said complexes from the beads with a buffer comprising 10% α -methyl mannoside.

101. (New) The composition of claim 65, further comprising an adjuvant.

102. (New) The composition of claim 101, wherein the adjuvant is selected from the group consisting of pluronic tri-block copolymer, muramyl dipeptide, detoxified endotoxin, saponin and liposome.

103. (New) The composition of claim 101, wherein the adjuvant is QS-21.

104. (New) The method of claim 66, 69, or 72, wherein the composition further comprises an adjuvant.

105. (New) The method of claim 104, wherein the adjuvant is selected from the group consisting of pluronic tri-block copolymer, muramyl dipeptide, detoxified endotoxin, saponin and liposome.

106. (New) The method of claim 104, wherein the adjuvant is QS-21.

107. (New) The composition of claim 65, wherein the human tumor cells are leukemic cells.

108. (New) The method of claims 66, 69, or 72, wherein the human tumor cells are leukemic cells.

109. (New) The composition of claim 107, wherein the leukemic cells are isolated from a human with myelogenous leukemia, monocytic leukemia, or lymphocytic leukemia.

110. (New) The method of claim 108, wherein the leukemic cells are isolated from a human with myelogenous leukemia, monocytic leukemia, or lymphocytic leukemia.